COLLECTION OF MATERIAL DNA SAMPLES FROM BIRDS IN THE GUNUNG HALIMUN NATIONAL PARK (GHNP) FOR ESTABLISHING OF DNA BANK

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ABSTRACT

This study was conducted to collect material DNA samples from birds in the Gunung Halimun National Park (GHNP) for establishing of DNA bank. Ciptarasa, Geger Hanjuang and Cikaniki village areas were chosen as sites for sample collections in the GHNP. In order to take the sample from birds, transect lines were established at each site where series of mist nets were operated. The captured birds, both live- and died-birds, were identified. For the living birds, blood and/or shed feathers were taken from the bird prior to releasing. In case of died birds, tissue and liver were collected. The collected samples were transported to genetic laboratory at Zoological Division, Researh Center for Biology (RCB)-LIPI (The Indonesian Institute of Science) and kept in 4 °C. In this collection, a total of 411 collected samples were obtained, coming from 25 families and 79 species.The 19-endemic birds were found in GHNP.

Key words: Biodiversity, birds, DNA-samples, Gunung Halimun National Park, blood, shed feather.

INTRODUCTION

Indonesia is one of the megadiversity countries in the world. It has at least 47 distinct natural ecosystems ranging from the ice fields and alpine meadows of Papua (fomerly Irian Jaya) to wide variety of humid lowland forests, from deep lakes to swallow swamp, and from the spectacular coral reefs to sea-grass beds and mangrove swamps. Indonesia is rich in species and possesses up to about 17% of the total number of species in the world. Approximately 12% of the world's mammals, 15% of all amphibians and reptiles, 17% of all birds and at least 37% of the world's fish are available in Indonesia. The Indonesian government has decided some places as conservation areas for protecting of richness on the biodiversities.

The forests of the Halimun is one of conservation areas in West Java, Indonesia (Surat Keputusan Menteri tertanggal 26 Februari 1992). The Gunung Halimun National Park is the only tropical rain forest which is the biggest and still remain in the Java Island. It is consisted of varieties ecosystem and cause rich biodiversity on flora and fauna, especially on endemic species, including wild and rare animals. In this National Park, the occurrences and general distribution of birds have been quite well studied (MacKinnon, 1993; UEA, 1994). MacKinnon (1993) listed 135 species of birds could be found in the Halimun area, while UEA (1994) listed 145 bird species observed in the area. This natural habiat need to be maintained for future use.

The megadiversity of genetic resources is not only important for Indonesia but it has been an international concern, since they may have importantly unknown genetic materials for future generations. However, Indonesia has the largest number of threatened species on birds and mammals (2000 IUCN Red List of Threatened species). In order to reduce the potential lost on the existing biodiversity in Indonesia, major efforts to conserve and preserve the natural habitat such as Gunung Halimun are under way through *in-situ* conservation, collection of living organisms as maintained in zoological parks and the amassing and documentation of museum specimen. However, relatively little effort has been made to collect and documenting DNA materials as genetic resources. With excellent techniques on DNA technology available, *ex-situ* conservation of the biological resources may be developed in the form of DNA bank. This approach is believed to be more important in the case of endemic and endangered species.

The availability of DNA bank for all birds classified as endemic and endangered are expected

to be a basis for further study in genetics using many different approaches from the simplest to the most expensive one. The existence of DNA Bank may also be opened to establish collaborative research with foreign counterparts whose results may be contributed to international community. With the described background above, an initial study to collect as many material DNA sample as possible from birds was conducted as an effort to establish DNA Bank in the Division of Zoology, Research Centre for Biology-LIPI (The Indonesia Institute of Sciences)..

METHODOLOGY

Study site

Three sites were chosen for collection of samples in this study:

1. Ciptarasa Village, Mountain Bodas areas, Sukabumi (876 m altitute). This village was visited during 10 days-survey on September 23rd to October 2nd, 1999.
2. Geger Hanjuang Village, Mountain Bengbreng, Sukabumi (1074 m altitute). This village was visited during 10 days-survey on September 23rd to October 2nd 1999.
3. Cikaniki Village, Sukabumi (1000 m altitute). Cikaniki village was visited every month for collection of samples. This activity has been started on January 2000 and a part of long term program for bird community in GHNP, specially around Cikaniki village areas. It was conducted monthly from January 2000 to December 2001, and has been conducted every 2 months since January 2002.

Collection of material DNA samples

Transect lines were established at each site where series of mist nets, up to 10 nets were operated. The nets used were 2.6 m high, 36 mm mesh size, and 12 m long. Nets were checked during daylight hours, once every hour. The bottom edge of the lowest net panel was generally set 0.3 m above the ground.

The captured birds, live and died birds were identified, and only the live birds were ringed. Samples were collected in the form of blood, shed feathers, liver or tissue. Blood and/or shed feathers were obtained from living birds after the birds were identified and ringed, prior to releasing the birds. In case of died birds, samples were obtained from tissue and liver.

Blood was taken from vena in the wing areas using 1 ml-syringe. Before the syringe used, it has to be rinsed with 10% EDTA. Total volume of blood was taken 0.05- 0.10 ml from individual of bird. After collecting samples, each sample was preserved with 96% of ethanol absolute in a 1.5 ml eppendorf tube and transported to genetic laboratory at Zoological Division, RCB - LIPI. These collected samples were kept in 4 °C refrigerator and ready for further processing in Laboratory.

Extraction and measuring concentration of DNA

This activity was done in Genetic Laboratory, Zoological Division, RC for Biology-LIPI, Cibinong, Indonesia. Total genomic DNAs of collected samples were extracted using a standard protocol (Sambrook *et al,* 1989).

All extracted samples were subjected to measurements of DNA concentration. It is important to quantify DNA molecules obtained for further analysis. There are two methods that are widely used to measure the amount of nucleic acid in preparations (Sambrook *et al,* 1989). In this study, the amount of nucleic acid was measured using a spectrophotometer (Beckman DU 650, Made in USA) based on the amount of ultraviolet irradiation absorbed by the bases and also was estimated from the intensity of fluorescence emitted by ethidium bromide.

The spectrophotometric readings of Optical Density (OD) was taken at wavelength of 260 and 280 nanometer (nm). The ratio between the reading at 260 nm ($OD_{26}o$) and 280 nm (OD_2g_0)

gives a measure of the purity of the DNA samples, with pure DNA having a ratio of 1.8. The OD_{260} allows calculation of the concentration of nucleic acid in the sample. An OD of 1 corresponds to approximately 50µg/ml for double stranded and 40µl/ml for single stranded DNA or RNA (Sambrook *et al.*, 1989).

Conversion of OD_{260} into a concentration unit (Hg/ml or ng/µl):

Concentration = OD_{260} X 50 X 50

Where A_{260} = value of OD_{260}
50 = a dilution factor
50 = value equivalent to 1 of OD_{260}

RESULTS

A summary of collected samples from Gunung Halimun National Park (GHNP) is presented in Table 1. Systematic arrangement used in this report generally followed Andrew (1992) and MacKinnon *et al.* (1992).

From the survey conducted on 23rd September to 2nd October, 1999 in Ciptarasa **and** Geger Hanjuang villages, 17 bird species coming from 8 families were collected. In fact, the 17 species found in Ciptarasa and Geger Hanjuang villages were also found in Cikaniki village. From this study, it was found more species in Cikaniki than in Ciptarasa and Geger Hanjuang areas. It can be summarized that the total material DNAs up to April 2002 were 411 samples. These collected samples are coming from 25 families and 79 species (see Table 1). Among these, 19 species are endemic birds (Mac Kinnon *et al.*, 1992 and see Table 2) and 22 species are protected birds by Indonesian Law (PP No.7 1999, see Table 3).

The biggest number of species found in GHNP is from the Muscicapidae family (12 species), followed by Timaliiday (9 species) and Turdidae families (8 species)). Because of limited time, not all of the samples (Table 1) have been extracted.

Protected birds including parrot and javan eagle are some of our target in further research. Eventhough they are inhabited in the GHNP, samples are not obtained yet. Efforts to get the sample are being continued.

Table 1. Collected samples from the GHNP for establishing of DNA bank

No	Family	Species	Total Samples	Form of sample
1.	Turdidae	1. *Erithacus cyane*	2	Blood
		2. Brachypteryx leucophrys	11	Blood, feather
		3. *Cinclidium diana*	13	Blood, feather
		4. *Zoothera adromedae*	12	Blood
		5. *Myophoneus glaucinus*	5	Blood, feather
		6. Enicurus velatus	12	Blood, feather
		1. Myophoneus caeruleus	2	Blood
		8. *Enicurus leschemulti*	2	Blood
2.	Pycnonotidae	1. *Alophoixus bres*	13	Blood, feather, liver
		2. Me virescens	4	Blood, feather
3.	Nectariniidae	1. *Arachnothera longirostra*	16	Blood, feather
		2. Arachnothera affinis	4	Blood
		3. *Anthreptes singalensis*	1	Blood
		4. *Anthreptes malacensis*	1	Blood
		5. *Aethopyga eximia*	2	Blood
		6. *Aethopyga mystacalis*	2	Blood
4.	Picidae	1. *Sasia abnormis*	9	Blood
5.	Timaliidae	1. *Malacocincla sepiarium*	11	Blood
		2. Pellorneum pyrrogenys	14	Blood, feather
		3. *Stachyris thoracica*	16	Blood, feather
		4. *Stachyris gramiceps*	12	Blood
		5. *Stachyris melanothorax*	9	Blood, feather, liver
		6. *Napothera epilepidota*	11	Blood, feather
		1. Napothera macrodactyla	3	Blood

Table J Continue

No	Family	Species	Total Samples	Form of sample
		8. *Pnoepyga pusilla*	7	Blood
		9. Pteruthius aenobarbus	8	Blood
6.	Cuculidae	1. *Cacomantis sepulcralis*	2	Blood
		2. Cuculus saturatus	2	Blood
7.	Dicruridae	1. *Dicrurus remifer*	6	Blood, feather
		2. Dicrurus annectans	2	Blood
8.	Muscicapidae	1 *Rhynomias olivacea*	18	Blood, feather.liver
		2. *Rhynomias brunneata*	1	Blood
		3. *Ficedula dumetoria*	13	Blood, feather
		4. *Ficedula hyperythra*	1	Blood, feather
		5. *Ficedula mugimaki*	7	Blood
		6. *Ficedula westermanni*	1	Blood
		7. *Muscicapaferruginea*	4	Blood
		8. *Rhipidura euryura*	8	Blood, feather
		9. *Culicicapa ceylonensis*	5	Blood
		10. *Eumyas indigo*	7	Blood
		11. *Cyornis banyumas*	4	Blood
		12. *Muscicapa danurica*	2	Blood
9.	Sittidae	1. *Sitta azurea*	11	Blood
10.	Podargidae	1. *Batrachostomus javensis*	1	Blood
		2. *Batrachostomus cornutus*	6	Blood
11.	Strigidae	1. *Otus refescens*	3	Blood
		2. *Otus lefnpiji*	2	Blood
		3. *Otusbrooki*	1	Blood
12.	Chloropseidae	1. *Chloropsis cochinchinensis*	6	Blood
		2. *Aegithina tiphia*	1	Blood
13.	Silviidae	1. *Phylloscopus trivirgatus*	3	Blood
		2. Gerygone sulphured	1	Blood
		3. *Abroscopus superciliaris*	8	Blood, tissue
		4. *Tesia superciliaris*	2	Tissue
		5. *Seicercus grammiceps*	1	Blood
		6. Acrocephalus orientalis	1	Blood
14.	Ploceidae	1. *Lonchura leucogastroides*	12	Blood
		2. Erythrura hyperrythra	3	Blood
		3. *Erythrura prasina*	2	Blood
15.	Eurylaimidae	1. *Eurylaimus javanicus*	2	Blood
16.	Zosteropidae	1. *Zosteros palpebrosus*	4	Blood
		2. Zosteros montanus	1	Blood
		3. *Lophozosterops javanicus*	1	Blood
17.	Plasianidae	1. *Arborophilajavanica*	4	Blood
18.	Meliphagidae	1. *Dicaecum trigonostigma*	8	Blood
		2. Dicaecum trochileum	2	Blood
		3. *Dicaecum sanguinolentum*	2	Blood
		4. Dicaecum cruentatum	1	Blood
19.	Dicaeidae	1. *Prionochilus percussus*	7	Blood
20.	Alcedinidae	*l.Alcedo euryzona*	4	Blood
		2. Lacedo pulchella	1	Blood
		3. *Halcyon cyanoventris*	1	Blood
21.	Campephagidae	1. *Pericrocotus miniatus*	10	Blood
22.	Trogonidae	1. *Harpactes oreskios*	1	Blood
		2. Harpactes reinwardtii	4	Blood
23.	Accipitridae	1. *Accipiter trivirgatus*	1	Blood
		2. Accipiter gularis	1	Blood
24.	Columbidae	1. *Macropigia unchall*	2	Blood
25.	Capitonidae	1. *Megalaima armillaris*	2	Blood

Table 2. Samples of endemic bird collected in GHNP

No	Species	Family	Endemic
1.	*Cinclidium diana*	Turdidae	Sumatra & Java
2.	*Enicurus velatus*	Turdidae	Sumatra, Java
3.	*Stachyris thoraicica*	Timaliidae	Java
4.	*Stachyris gramiceps*	Timaliidae	Java
5.	*Stachyris melanothorax*	Timaliidae	Java & Bali
6.	*Rhipidura euryura*	Muscicapidae	Java
7.	*Eumyas indigo*	Muscicapidae	Sunda Besar
8.	*Batrachostomus cornutus*	Podargidae	Sunda Besar
9.	*Tesia superciliaris*	Silviidae	Java
10.	*Seicercus gramiceps*	Silviidae	Sumatra, Java, Bali
11.	*Arborophilajavanica*	Plasianidae	Java
12.	*Pericrocotus miniatus*	Campephagidae	Java & Sumatra
13.	*Aethopyga eximia*	Nectariniidae	Java
14.	*Aethopyga mystacalis*	Nectariniidae	Java
15.	*Lophozosterops javanicus*	Zosteropidae	Java, Bali
16.	*Halcyon cyanoventris*	Alcedinidae	Java
17.	*Megalaima armillaris*	Capitonidae	Java & Bali
18.	*Harpactes reinwardtii*	Trogonidae	Java & Sumatra
19.	*Dicaecum sanguinolentum*	Meliphagidae	Java & Lasser Sundas

Table 3. Samples of protected birds obtained in GHNP

No.	Species	Family	Indonesian Law
1.	*Arachnothera longirostra*	Nectariniidae	PP No. 7 1999
2.	*Arachnothera affinis*	Nectariniidae	PPNo.7 1999
3.	*Anthreptes singalensis*	Nectariniidae	PP No. 7 1999
4.	*Anthreptes malacensis*	Nectariniidae	PPNo.7 1999
5*.	*Aethopyga eximia*	Nectariniidae	PPNo.7 1999
6*.	*Aethopyga mystacalis*	Nectariniidae	PPNo.7 1999
7*.	*Stachyris gramiceps*	Timaliidae	PPNo.7 1999
8*.	*Stachyris melanothorax*	Timaliidae	PPNo.7 1999
9*.	*Rhipidura euryura*	Muscicapidae	PPNo.7 1999
10*.	*Lophozosterops javanica*	Zosteropidae	PP No.7 1999
11.	*Alcedo euryzona*	Alcedinidae	PPNo.7 1999
12.	*Lacedo pulchella*	Alcedinidae	PPNo.7 1999
13*.	*Halcyon cyanoventris*	Alcedinidae	PPNo.7 1999
14.	*Harpactes oreskios*	Trogonidae	PPNo.7 1999
15.	*Harpactes reinwardtii*	Trogonidae	PPNo.7 1999
16.	*Accipiter trivirgatus javanicus*	Accipitridae	PPNo.7 1999
17.	*Accipiter gularis*	Accipitridae	PPNo.7 1999
18*.	*Megalaima armillaris*	Capitonidae	PPNo.7 1999
19.	*Dicaecum trigonostigma*	Meliphagidae	PPNo.7 1999
20.	*Dicaecum trochileum*	Meliphagidae	PP No.7 1999
21*.	*Dicaecum sanguinolentum*	Meliphagidae	PP No.7 1999
22.	*Dicaecum cruentalum*	Meliphagidae	PPNo.7 1999

Note: *endemic and protected birds.

DISCUSSION

Table 1 shows total number of collected samples from GHNP were 79 species. All species of birds found in this study were also observed in Adhikerana's study (1998). This is equal to 5.13% of the Indonesian birds (Andrew, 1992). Eventhough the 79 species has been conducted for more than two years, but the total species have been observed from GHNP was less than the total species listed by Mac Kinnon (1993) and UEA (1994). At the moment, 58.5 % (MacKinnon, 1993) or 54.5% (UEA, 1994) of the total birds in GHNP are available in the form of material DNA. Therefore, there are still a lot more of material DNA samples from other species in GHNP need to be collected for DNA bank. The important results

from the collected samples included 19 species of endemic birds and 22 species of protected birds by Indonesian Law. This valuable collection, especially for endemic species is a big potential of genetic recourses to establish wider collaborative research with international researchers.

Samples listed in the Tables 1 were screened based on the conservation status for the priority of research and these collected material DNA are very valuable. They will be a potential source for DNA bank. In the near future, the availability of the genomic DNA allow us to make DNA bank. This will be a very important step for further studies since the genetic recourses provide essential tool facilitating scientific research. Some important point in the existence of DNA bank for genetic studies are to enrich understanding of evolution, to determine single ancestor of species through molecular analysis, to study genetic diversity and to conduct forensic study.

Under Pro Natura Fund, establishment of DNA bank on mammal and birds has been started in Zoological Division, RC for Biology-LIPI since 1999. Since then, other positive activities are acummulatively being gained. More and more new DNA collection are deposited, for example, this recent activity on material DNA collection of birds from GHNP has being conducted since 2000. Other positive impact of those activities is that researcher in Division of Zoology who has collaborative study with other overseas and domestic researcher donated his/her DNA sample to be collected in the Division of Zoology. Expectedly number of DNA collection at the Zoological Division will be accumulated from time to time. In the future, availability of complete DNA samples representing Indonesian fauna will make the division a center for DNA collection in Indonesia.

CONCLUSSION

The 19- endemic birds found in GHNP are important species in term of conservation. Results observed from this initial effort may be useful for further genetic studies involving collaboration between Indonesia and Japanese institutions or other worldwide.

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REFERENCES

Adhikerana AS, Komeda S, Wijamukti S and Marakarmah A. 1998. The Birds of Mountain Halimun National Park, "West Java: with Particular References to Altitudinal Distribution. Dalam: *Research and Conservation of Biodiversity in Indonesia,* **Volume IV,** *Mountain Halimun: The Last Submontane Tropical Forest* In West Java (Simbolon *et al.* Eds). A Joint Project with LIPI, PKA and JICA.

Andrew P. 1992. *The Birds of Indonesia. A* Checklist (Peters Sequence). Kukila Checklist No.l. Indonesian Ornithological Society, Jakarta.

Craig Hilton-Taylor, 2000. *IUCN Red List of Threatened Species.* IUCN - The World Conservation Union.

MacKinnon J, 1993. *A Field Guide to the Birds of Java and Bali.* Gadjah Mada University Press, Yogyakarta.

MacKinnon J, Phillipps K and Balen Bas Van. 1992. *A Field Guide to the Birds of Sumatera, Java, Bali and Kalimantan.* Puslitbang Biologi - LIPI.

Sambrook J, Fritsch EF and Maniatis T, 1989. *Molecular Cloning: A Laboratory Manual.* 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York.

UEA (University of East Anglia). 1994. Biodiversity Study og Mountain Halimun National Park. **Study Report.**